MICROBIAL ANALYSIS OF DEPOSIT DUST ON SURFACES IN BUILDINGS OFFICES EQUIPPED WITH CENTRAL AIR-CONDITIONING INSTALLATIONS: PROPOSED MICROBIAL PRACTICAL VALUES WITH A NEW STANDARDIZED METHOD

C Chasseur*, S Gofflot, N Nolard

Scientific Institute of Public Health - Mycology Section -14, rue Juliette Wytsman B-1050 Brussels (Belgium)

ABSTRACT

A method for monitoring the microbiological composition of deposit dust in buildings equipped with HVACs (Heating, Ventilation, and Air Conditioning) is described. A piece of glass, firstly washed with an alcohol solution was placed on furniture for 7 days. Afterwards, 5 RODAC plates, were applied to the glass, and incubated at appropriate temperatures. From July 1999 to August 2000, more than 180 samples were made in 19 buildings for bacteria and in 24 buildings for moulds. Total bacteria and total moulds were counted. Main moulds were identified. Values situated between centile 50 and 75 were considered as a good practical range of values to determine the "average conditions" of different taxa: 18 to 34 CFU/plate for mesophilic environmental bacteria (25°C), and 14 to 28 CFU/plate for mesophilic human-source bacteria (37°C), 12 to 23 CFU/plate for mesophilic moulds. Thermophilic moulds (45°C), and thermophilic Actinomycetes (52°C) were rarely isolated.

INDEX TERMS

Surface analyses, RODAC, Microbial quality scale, HVAC

INTRODUCTION

In order to evaluate the microbiological level of indoor air (IDA) various methods are generally used. Air analysis is generally the first approach but requires expensive samplers, i.e. Andersen, RCS, SAS, MAS (Flanigan, 1997 and ACGIH, 1999). Moreover, air samples are generally taken in a fairly short time, which always presents the risk of missing a specific contaminant. It was thus interesting to propose a complementary technique, simple and inexpensive, which makes it possible to collect information during 7 days. Here are some of the main methods used for surface microbial examination: the swab-rinse technique, developed in 1917 by Manheimer and Ybanez, the acetate adhesive tape technique (Edwards, 1951; Edwards, Hartman, 1952), the rinse technique (Clarck, 1965 and Favero 1968) and the direct surface agar plating (RODAC) (Angelotti and Foter, 1958; Hall, and Hartnett, 1964; Whyte, Carson, and Hambraeus, 1988; Favero, 1968, and Pitzura et al., 1997). We chose and adapted the RODAC plate method, standardizing the support examined where dust has settled (the airborne dust collector); the sampling period was set at 7 days. The microbiological spectrum was increased with 5 different media and incubation temperatures. For bacteria, we referred to Otten and Burge (ACGIH, 1999) who refer to the following basic distinction between mesophilic bacteria including environmental

* Contact author email: C.Chasseur@iph.fgov.be

bacteria(18 to 30°C) and human sources bacteria (35 to 44°C), from thermophilic bacteria (50 to 55°C) including *Thermoactinomyces.*

METHODS

The airborne dust was collected on a piece of glass (20cm x 20cm), washed beforehand with an alcohol solution, and generally left on furniture in buildings. After 7 days, a surface sample is taken on the glass with a kit of 5 RODAC plates (23.74 cm^2) filled with different specific agar media (MEA+chloramphenicol for moulds, TSA for bacteria, TSA+novobiocine for Actinomyces). This kit allows for the recovery of a large spectrum of germs (mesophilic (25°C) and thermophilic (45°C) moulds, thermoactinomyces (52°C), total mesophilic bacteria at 25 and 37°C), from different sources (Chasseur, 2000). Plates are applied to the glass surface with an automatic applicator at a pressure of 25 gr/cm^2 for 10 seconds (ISO DIS 14698-1) and are then incubated in laboratory at the appropriate temperature. From July 1999 to August 2000, 189 samples were taken with this method in buildings equipped with HVAC, 19 for bacteria and 24 for moulds.

RESULTS AND DISCUSSION

1. Validation step

A first experiment was made to evaluate glass cleaning with alcohol. In order to do this, 10 pieces of glass cleaned with alcohol and 10 others autoclaved were checked at the beginning of the experiment. The results obtained are presented in Table 1. Even though the results are better with autoclaved glass, for in this type of environment alcohol cleaning is sufficient.

Table 1. Examination of surfaces at the beginning of the experiment (t_0). Comparison between cleaned and sterilized pieces of glass (10 repetitions, mean, SD)

	Units	Bacteria 25°C	Bacteria 37°C	Actino 52°C	Moulds 25°C	Moulds 45°C
Cleaned with alcohol						
Mean ± SD	*CFU/23,74cm²*	1 ±1	1 ±1	0	0	0
Range	*CFU/23,74cm²*	0-2	0-3	0	0	0
Autoclaved						
Mean ± SD	*CFU/23,74cm²*	0	0	0	0	0
Range	*CFU/23,74cm²*	0	0	0	0	0

A second experiment estimated the precision of the method. series of 10 pieces of glass were placed in offices, and checked after 7 days: in an environment both without HVAC (2 series), rather overcrowded and enclosed, and with HVAC (1 series). Table 2 shows that the results for bacteria incubated at 37°C, the coefficient of variation (CV), varied from 9 to 20%, and for bacteria incubated at 25°C, from 10 to 27% For mesophilic moulds, CV varied from 22 to 28%. These variations may be due to the difficulties of counting when colonies are too numerous (confluence) or when colonies are overlapped by an invasive species (overrunning). Moreover, dust deposit on glass may not always be totally uniform.

Table 2. Examination of surfaces after 7 days (t_7)

	Units	Bacteria 25°C	Bacteria 37°C	Actino. 52°C	Moulds 25°C	Moulds 45°C
1. Office without HVAC						
Series 1						
Mean ± SD (n=10)	*CFU/23,74cm²*	93 ±9	110 ±10	0	57 ±16	0
CV	*%*	10	9	/	28	/
Range	*CFU/23,74cm²*	81-104	93-124	0	27-82	0
Series 2						
Mean ± SD (n=10)	*CFU/23,74cm²*	125±36	179±22	0	27±6	2±2
CV	*%*	29	12	/	22	/
Range	*CFU/23,74cm²*	68-170	150-221	0	13-32	0-5
2. Office with HVAC						
Mean ± SD (n=10)	*CFU/23,74cm²*	30±8	25±5	0	2±2	0
CV	*%*	27	20	/	/	/
Range	*CFU/23,74cm²*	21-44	18-30	0	0-5	0

2. Evaluation of total germs

Evaluation of total indoor germs in various equivalent buildings equipped with HVAC allowed us to evaluate the "microbial background". In Table 3, various percentiles were calculated, which may serve as a quality reference scale to evaluate the microbial level of the working place examined, in terms of cleanliness requirements. Results between centile 50 and 75 were considered as good practical values to determine the "average conditions" of different taxa:18 to 34 CFU/plate for mesophilic environmental bacteria (25°C), and 14 to 28 CFU/plate for mesophilic human-source bacteria (37°C).

Total bacteria incubated at 25°C may generally be considered as coming from outside environments or inside a contaminated environment (water of humidifier for instance). We may also consider that a large proportion of the total sampled bacteria incubated at 37°C came from human sources. These germs are thus often found in large quantities in overcrowded and enclosed indoor environments.

Table 3. Results of surface examination in varied working places equipped with air-conditioning (total initial samples=193)

Centiles	**Appreciation**	**Bacteria 25°C**	**Bacteria 37°C**	**Moulds 25°C**	**Moulds 45°C**	**Actino. 52°C**
$<C_{25}$	very low	8	5	6	0	0
C_{25}-C_{50}	low	9 - 17	6 - 13	7 - 11	0	0
C_{50}-C_{75}	**average**	**18 - 34**	**14 - 28**	**12 - 23**	**0 - 1**	**0**
C_{75}-C_{95}	high	35 - 81	29 - 85	24 - 51	2	0
$>C_{95}$	very high	>82	>85	>51	>2	0
Range		0 - 193	0 - 146	0 - >69	0 - 58	0 - 0
Total samples		190	192	186	143	143

For total thermophilic actinomycetes and thermophilic moulds the 143 samples examined were all negative. But in practice, even though contamination is rare, it exists. Thermophilic actinomycetes may contaminate warm coils, generally located too close to a humidifier, and *Aspergillus fumigatus* is sometimes isolated in large quantities on pulsion fans or on walls inside air ducts. The mere presence of these taxa requires further investigation.

For total mesophilic moulds (25°C), the average condition was characterised by a range of values from 12 to 23 CFU/plate, values which should be moderated according to taxa diversity and specificity.

3. Moulds taxa

A large variety of fungal species can be found in indoor air. Most of them are generally among the most common outside species which may come in from outside through windows, doors or the air-conditioning system when filters are defective. Their presence in large quantities in indoor air does not necessarily mean that a microbial contamination exists in the building. Some of them are strictly parasites of plants (*Cladosporium herbarum, Botrytis cinerea*), and identification gives precious information about the origin. It is well known, that when an important indoor concentration, for instance of *Cladosporium is* found, a high level of *C. herbarum* means an outside origin, whereas a high level of *C. sphaerospermum* or *C. cladosporioides* means an indoor contamination (indoor amplification). The parasitic characteristic of *C. herbarum* implies that it cannot have a saprophytic development on wet walls in buildings or in humidifier. It is the same for a lot of moulds (*Botrytis cinerea,* …)
When examining the taxa occurrence (% of plates with presence of one concerned species), 2 groups can be distinguished, one with high occurrence and another with low occurrence (Table 4).

Table 4. Percentages of plates with presence of one concerned mesophilic taxa (occurrence)

n=186	Occurrence (%)		Occurrence (%)
Total *Penicillium*	**60**	*Cladosporium herbarum*	40
Total *Aspergillus*	**25**	*Cladosporium sphaerospermum*	4
Total *Cladosporium*	**76**	*Cladosporium cladosporioides*	10
Total *Alternaria*	**13**	*Fusarium spp.*	1
		Mucor spp.	2
Acremonium sp.	2	*Paecilomyces spp.*	4
Aspergillus flavus	4	*Phoma sp.*	2
Aspergillus fumigatus	9	*Rhizopus sp.*	2
Aspergillus nidulans	1	*Trichoderma sp.*	2
Aspergillus niger	1	*Ulocladium sp.*	4
Aspergillus ochraceus	4	Yeasts	13
Aspergillus versicolor	3	White sterile mycelia	7
Aureobasidium spp.	3	Grey sterile mycelia	2
Botrytis cinerea gr.	30	Other sterile mycelia	69
Chaetomium spp.	2	Other	8

Five genera presented a high occurrence, which is the same trend as the results obtained with airborne analyses by (McGrath et al., 1999), and by (Burges et al., 2000): *Penicillium* (60%) and

Aspergillus (25%), both belonging to Aspergillaceae, and *Cladosporium (76%), Alternaria (13%)* and *Botrytis (30%)*. Yeasts and non-identified sterile mycelia are also often represented in plates. These taxa are generally an important part of the permanent background of microorganisms in our environment, but high levels of these taxa should be considered a health hazard (Ahearn et al., 1997; McGrath et al., 1999; Gravesen, et al. 1999, Burge et al., 2000).

In 99% of cases (centile 0.99), the results did not exceed 1 or 2 CFU/plate. So for taxa with low occurrence, recommendations are made according to the specificity of isolated species. In some cases, the mere presence of 1 CFU/plate might require the greatest attention (*Stachybotrys chartarum* for its toxicity or *Chaetomium* for its allergenicity).

CONCLUSION

This method presents the following advantages: standardized sampling methods; information on a period of 7 days; more controlled information about the history of the sampled surface. Nevertheless, some questions remain: For instance, do all the taxa collected on glass have the same resistance to dryness ? Can they all be revived on selected media ? (Macher, 2000) considers that concentration of culturable bacteria and fungi in house dust doesn't vary at room temperature for up to 25 days. However, a better knowledge of the technique efficiency would improve the interpretation of the results.

We also should precise that this scale is not directly correlated with health problems. It is an evaluation with an easy method of the microbial occurrence in several buildings. Different centiles were calculated to establish a "quality scale", to compare the biological conditions of different places in buildings with HVAC. It is mainly a tool for technicians to improve cleaning or maintenance process. So these limits should be considered as "practical values". In practice, it is important to have at our disposal, different "action thresholds" which allows rapid and appropriate recommendations.

Moreover, when results are very high, and especially for specific germs, this method allows to evaluate some health risks. But of course, these risks are more relevant on the basis of the identification of isolated species than by these proposed values (presence of high concentration of *Penicillium, Stachybotrys chartarum, Aspergillus fumigatus,* …).

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